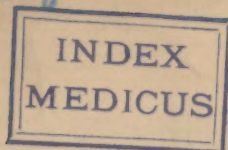


Wiley (H. W.)



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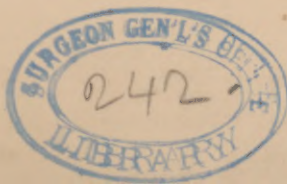
DETERMINATIONS OF LACTOSE IN MILKS BY OPTICAL METHODS.

BY HARVEY W. WILEY.

The usual method of determining milk sugar by evaporating the sample to dryness, and extracting the sugar with alcohol after exhausting with ether, requires a great deal of time and labor. If some reliable optical method could be devised, the determination of the lactose in milk would be the work of only a few minutes. The difficulties which are encountered in seeking for such a method are numerous and serious, so much so that little credit has heretofore been given to any of the processes of optical analysis in use. In the following paper I will give the results which have been obtained under my direction in the study of this problem. The optical work collected in the following tables was done chiefly by my assistants, Messrs. G. L. Spencer and C. A. Crampton; the alcohol extractions were made by Mr. A. E. Knorr, and the nitrogen estimation by Messrs. Fuller and Trescott.

Specific Rotatory Power of Milk Sugar.

Crystallised milk sugar when first dissolved possesses a higher rotatory power than it has in the milk from which it was derived. This increased optical activity may be compared with the original by the ratio 8 : 5, nearly. After the solution has stood for twelve to twenty hours, or immediately on boiling it, this extra rotatory



power is lost. In estimating the specific rotatory power of milk sugar the numbers given always refer to the constant and not the transient gyrotory property.

Among the earliest numbers assigned to the rotation of lactose are those of Poggiale $(a)_D = 54.2$ and Erdmann $(a)_D = 51.5$, (Sucrose $(a)_D = 66.5$). Biot¹ places this number for lactose at 60.23, and Berthelot² at 59.3 for the transition tint $(a)_l$. Hoppe-Seyler, in his "Handbuch der physiologisch-chemischen Analyse," gives this number at $(a)_l = 58.2$. Since the ratio of $(a)_D$ to $(a)_l$ is 1 : 1.1306, the above numbers become for Biot $(a)_D = 53.27$, for Berthelot $(a)_D = 52.47$, and Hoppe-Seyler $(a)_D = 51.48$. Hesse³ observed the rotation number to be $(a)_D = 52.67$, when the solution contained 12 g. per 100 cc. and the temperature was 15°. On the other hand, when the concentration is only 2 g. per 100 cc. the number assigned is $(a)_D = 53.63$. It appears from this that the specific rotation power of a solution of milk sugar diminishes with the increase of its concentration, and this view is adopted by Landolt, Tollens and Schmidt.

The following general formula⁴ is used to correct the reading of the polariscope for concentration of solution:

$$(a)_D = 54.54 - .5575 c + .05475 c^2 - .001774 c^3,$$

in which c = number grams sugar in 100 cc. solution. These observations are contradicted by the work of Schmoeger,⁵ who, in an elaborate series of experiments, using instruments of different construction and observing all necessary precautions, found the rotation number of lactose sensibly constant for all degrees of concentration up to the saturation point. In 32 series of investigations, in which the degree of concentration gradually increases from $c = 2.3554$ to $c = 36.0776$, and in which a constant temperature of 20° was maintained, the variations in the numbers obtained were always within the limits of error of observation. The mean of all these numbers fixes the value of $(a)_D$ at 52.53.

According to Schmoeger, variations in temperature have far more to do with changes in rotatory power than differences of concentration. The value of $(a)_D$ falls as the temperature rises.

¹ Compt. rend. **42**, 349.

² Dict. de Chem. par Ad. Würtz 2, 2d part, p. 188.

³ Annal. Chem. u. Pharm. **176**, 98.

⁴ Tucker, Sugar Analysis, 91.

⁵ Berichte der deutsch. chem. Gesell. 1880, 1922 et seq.

Under 20° the disturbing influence of temperature is greater than above 20° . At the latter degree $(a)_D$ varies inversely about .075 for each 1° change of temperature. Pellet and Biard,¹ as a result of their observations, fix the rotatory power of milk sugar at 58.94 for $(a)_D$ ($(a)_D = 52.12$).

After a careful review of the methods used in the above *résumé* and the numbers determined by them, I am inclined to accept the mean obtained by Schmoeger as the one entitled to the greatest credit. It also has the advantage of being almost the mean of all the various numbers which have been assigned as the specific rotating power of lactose, viz.,

Poggiale,	.	.	.	54.2
Erdmann,	.	.	.	51.5
Biot,	.	.	.	53.27
Berthelot,	.	.	.	52.47
Hoppe-Seyler,	.	.	.	51.48
Hesse,	.	.	.	52.67
"	.	.	.	53.63
Schmoeger,	.	.	.	52.53
Pellet and Biard,	.	.	.	52.12

Mean, 52.65

In the present state of our knowledge, therefore, the specific rotatory power of milk sugar should be taken at $(a)_D = 52.5$. I propose, at an early date, to make a careful study of this subject, in order to fix, if possible, an exact number for the expression of the rotating power, and to examine the conflicting evidence respecting the influence of the degree of concentration on the same. The estimation of lactose in milk by the polariscope is rendered difficult also by the presence in milk of various albumens—all of which turn the plane of polarisation to the left. As will be seen by the data given further along, the ordinary method of removing these albumens, viz. by a solution of basic lead acetate, is far from being perfect. If, therefore, a portion of the albumen be left in the liquid submitted to polarisation, the rotation to the right will be diminished by its presence.

Hoppe-Seyler² assigns as the rotation power of egg albumen $(a)_D = -35.5$, and for serum albumen $(a)_D = -56$. Both acids

¹ Bull. de l'Assoc. des Chimistes 1, 5, 171 et seq. ² Würtz, Dictionnaire de Chimie 1, 91.

and alkalis seem to increase the rotating power, which may with acetic acid reach $(a)_D = -71$.

Fredericq¹ gives the rotation number for blood serum for the rabbit, cow and horse at $(a)_D = -57.3$, and for the dog at -44 . Paraglobulin, according to the same author, has a rotation number $(a)_D = -47.8$.

Milk albumen² has the following numbers assigned to it:

Dissolved in MgSO_4 sol.	$(a)_D = -80$
“ “ dil. HCl .	“ $= -87$
“ “ dil. NaOH sol.	“ $= -76$
“ “ strong KOH sol.	“ $= -91$

The hydrates of albumen³ have rotation powers which vary from $(a)_D = -71.40$ to $(a)_D = -79.05$. From the chaotic state of knowledge concerning the specific rotating power of the various albumens, it is impossible to assign any number which will bear the test of criticism. For the purposes of this paper, however, this number may be fixed at $(a)_D = -70$ for the albumens which remain in solution in the liquids polarised for milk sugar.

The phenomenon of “birotation” in milk sugar has already been noticed. The problem of analysis of this sugar is, however, still further complicated by the facts pointed out by Schmoeger⁴ and Erdmann⁵ that when milk is rapidly evaporated in a plain dish the sugar is left in the anhydrous state, and that this sugar in fresh solutions exhibits the phenomenon of “half rotation.” When such sugar is extracted with alcohol and re-evaporated, it, doubtless, is still anhydrous. But in the calculation of results this sugar is generally estimated as containing water of crystallisation, and thus an error, which Schmoeger reckons at as much as .2 per cent., is introduced into the results. This fact, not well recognised, combined with the knowledge that in the process of evaporation many particles of sugar must be occluded by the hardening caseine, tends to throw doubt upon the accuracy of estimating the sugar by the extraction method.

The work which I undertook had for its object the determination of the best method of preparing the milk-sugar solution for

¹ Compt. rendus, 93-465.

² Hoppe-Seyler in Handbook of the Polariscopes, Landolt, p. 248.

³ Kühne and Chittenden, Am. Chem. Journal 6, 45.

⁴ Ber. d. deutsch. chem. Gesell., 1880, 1915 et seq.; 1881, 212 et seq.

⁵ Ber. d. deutsch. chem. Gesell., 1880, 2180 et seq.

the polariscope, and a comparison of the numbers obtained by this instrument with those given by the ordinary process of extraction.

The reagents used for removing the albumens were :

- (1) Saturated solution basic lead acetate, sp. gr. 1.97.
- (2) Nitric acid solution of mercuric nitrate diluted with an equal volume of water.
- (3) Acetic acid, sp. gr. 1.040, containing 29 per cent. $\text{HC}_2\text{H}_3\text{O}_2$.
- (4) Nitric acid, sp. gr. 1.197, containing 30 per cent. HNO_3 .
- (5) Sulphuric acid, sp. gr. 1.255, containing 31 per cent. H_2SO_4 .
- (6) Saturated solution sodium chloride.
- (7) Saturated solution magnesium sulphate.
- (8) Solution of mercuric iodide in acetic acid ; formula¹ KI, 33.2 g. HgCl_2 , 13.5 g.

Strong $\text{HC}_2\text{H}_3\text{O}_2$,	20.0 cc.
Water,	640 cc.

Alcohol, ether and many solutions of mineral salts, hydrochloric and other acids were also tried as precipitants for albumen, but none of them presented any advantages which would make a detailed account of the experiments of any interest.

TABLE NO. I.

This table contains a record of the experiments which led to the adoption of 1 cc. acetate of lead solution, or 1 cc. acid mercuric nitrate, as the best amount of each for 50 cc. of milk.

Nearly all the polarisations were made in a 400 mm. tube. From two to four observations were made with each sample. An average of these readings was taken for each determination. In the calculations the value of $(a)_D$ was taken at 53 instead of 52.5, the number which subsequent investigations have led me to believe more exact. The instrument employed was a "Laurent Large Model" polariscope.

In all cases the volume of the solution was corrected for the volume of the precipitated caseine. This volume was assumed to occupy 2 cc. for each 50 cc. milk.

Since in the Laurent instrument the weight of sucrose in 100 cc. to read even degrees on the scale is 16.19 g. [$(a)_D = 66.67$], it follows that the weight of lactose in 100 cc. to read one degree on the

¹Journal de Pharmacie et de Chimie, Aug. 1884, 108.

TABLE I.

Reagents Employed in Precipitating Albumens.

No.	Per cent. lactose extracted by alcohol.	Per cent. Lactose.					Other Reagents.
		Pb 1 cc.	Pb 2 cc.	Pb 3 cc.	Pb 4 cc.	Pb 5 cc. A 5 cc.	
1	4.57				{ 10 cc.	3.67	4.23
2	4.52				{ 2.45	3.57	4.14
3	4.46	4.48					3.57
4	3.92	4.19				3.35	
5	4.35		3.55				4.32
6	3.71	4.01					3.00
7	4.10	{ 4.63					{ 4.44
		{ 4.96 H					{ 4.68 H
8	4.16	{ 4.29					{ 3.80
		{ 4.33					{ 3.67 H
9	4.48	{ 4.59					{ 4.04
		{ 4.58 H					{ 4.12 H
10	4.10	4.12 H					3.47 H
11	4.80	4.87 H			(4 cc.)	4.44 H	4 cc. 4.70 H
12	4.77	5.02 H	4.82 H	4.50 H	4.31 H		5 cc. 4.76 H
							6 cc. 4.76 H
							8 cc. 4.78 H
13	4.25	4.25	3.75	3.38	3.38		3.97
14	4.22	4.90 H	4.58 H				3.89
		{ 4.40					HNO ₃
15	3.14	{ 4.32 H					4.68 H
16	3.30	4.43 H					4.66 H
17	4.72	4.45	4.18	3.87	3.65	3.26	4.50 H
							3.96
							{ 3.98
							{ 3.88 H
18	4.88	{ 4.87					4.27
		{ 4.87 H					4.25
19	4.31	4.71					(5 cc.) 4.43 H ₂ SO ₄
		4.86 H					4.51
							4.59
20	4.39	4.11					4.79 H
21	4.70	4.17					
22	4.96	{ 4.93					{ 4.45
		{ 4.97 H					{ 4.43 H
		{ 4.41					{ 3.86
23	4.60	{ 4.45					{ 3.86
		{ 4.41					{ 3.90
		{ 4.45 H					{ 3.90
24	4.74	{ 4.33					{ 4.21
		{ 4.37 H					{ 4.32
		{ 4.33					{ 4.32
25	4.59	{ 4.37 H	{ NaCl	1 cc. MR			{ 4.35 H
		{ 4.29 H					{ 4.44 H
26	4.39			4.29 H			{ 4.03
27	4.60	4.18 H		4.66			{ 4.10 H
28	4.26	3.67 H		4.09			{ 3.98
							{ 4.10 H

scale for each per cent. lactose present would be 16.19, $x = 53$; 66.67, $x = 20.37$.

If 52.5 be taken as the value of $(\alpha)_D$ for lactose, then $x = 20.56$.

In table No. I, A indicates acetic acid, Pb basic acetate of lead, MR acid mercuric nitrate, etc. The letters C and H indicate the temperature; C denoting the ordinary temperature of the room, and H that the sample was heated to 100° and cooled before filtering.

The numbers obtained by extraction with alcohol are taken as the basis of comparison, not because I believe them to be more reliable, but because that method is the one generally employed in the estimation of milk sugar.

In the alcohol extraction the milk was evaporated to dryness in a thin glass capsule, the dish and dried residue pulverised in a mortar, washed with ether into a continuous extraction apparatus, exhausted with ether, and then with 80 per cent. alcohol for ten hours.

Duplicate analyses are indicated in the table by the small brackets.

Remarks on Table I.

The results obtained by using various other reagents for the precipitation of the caseine, viz. $MgSO_4$, $CuSO_4$, HCl , etc., have not been entered in the table. In none of these cases was there sufficient encouragement to warrant an extended trial. In most cases the precipitation was slow or imperfect, and the filtration difficult.

One important fact should not be overlooked, viz. that any excess of basic plumbic acetate causes a rapid decrease in the rotatory power of the solution; whether this decrease is due to precipitation of the sugar or solution of the albumens does not clearly appear. Illustrations of this decrease are seen in analyses 2, 12, 13 and 17.

It seems to make little difference whether the precipitation is made hot or cold. The question of temperature is set forth in greater detail in the next table. From all the experiments made it clearly appeared that the best optical results are obtained by the use of a minimum quantity of basic lead acetate, or of either the acid mercuric nitrate or iodide. For 50 to 60 cc. of milk, 1 cc. of the lead acetate or mercuric nitrate solution of the strength noted,

and 25 cc. of the mercuric iodide solution are the proper quantities. It makes no difference, however, if a large excess of the two latter reagents is employed. Of the three the last is to be preferred.

TABLE NO. II.

In this table will be found the results of the comparative determinations of milk sugar by extraction with alcohol, by precipitation with 1 cc. basic lead acetate, and the same with 1 cc. acid mercuric nitrate, hot and cold, to each 60 cc. of milk.

In many of the analyses the large differences in results by the three methods show a fault of manipulation, but all the results have been given without selection.

TABLE II.

No.	Reagents Employed.				
	Extract by Alcohol.	C. Pb 1 cc.	H. Pb 1 cc.	C. MR 1 cc.	H. MR 1 cc.
Percentage of Milk Sugar.					
1	4.55		4.74		4.92
2	4.10		4.22		4.50
3	4.51	4.54	4.22	4.68	4.62
4	4.36	4.55	4.53	4.89	4.90
5	4.05	4.14	4.09	4.48	4.39
6	3.84		3.84	3.98	3.98
7	4.52	4.67	4.73	5.01	5.00
8	4.25	4.21	4.26		4.51
9	4.45	4.61	4.54	4.87	4.87
10	4.92	5.20	5.22	5.43	5.47
11	3.84		3.72	4.00	3.96
12	4.53	4.61	4.64	4.87	4.85
13	4.57	4.54	4.55	4.91	4.84
14	4.66	4.29	4.45		4.63
15	4.17	3.65	3.75	3.95	3.87
16	5.02	4.66	4.64	4.86	4.86
17	4.68	4.03	3.94	4.39	4.37
18	4.23	3.82	3.89	4.08	4.02
19	4.96	4.70	4.84	5.04	5.04
20	4.85	4.39	4.41	4.53	4.65
21	4.63	4.47	4.47	4.69	4.67
22	4.47	4.39	4.45	4.67	4.71
23	4.46	4.23	4.31	4.65	4.63
24	4.47	4.59	4.67	5.01	4.95

No.	Extract by Alcohol.	C. Pb 1 cc.	H. Pb 1 cc.	C. MR 1 cc.	H. MR 1 cc.
25	4.40	4.41	4.55	4.45	
26	4.85	4.67	4.73	4.97	
27	4.45	4.21	4.33	4.57	
28	4.44	3.98	4.10	4.28	
29	4.10	4.21		4.55	
30	4.38	5.57	4.69	4.89	
31	4.20	4.21	4.37	4.57	
32	4.69	4.59	4.67	4.89	
33	4.52	4.27	4.41	4.41	
34	4.37	4.65	4.93	4.93	
35	4.52	4.27	4.41	4.56	
36	4.88	4.83	4.93	5.17	
37	4.61	4.30	4.43	4.57	
38	4.79	4.59	4.67	4.91	
39	4.67	4.26	4.41	4.51	
40	4.79	4.64	4.74	4.94	
41	3.95	4.10	4.26	4.38	
42	4.00	4.61	4.61	4.77	
43	4.63	4.24	4.37	4.57	
44	4.77	4.64	4.70	4.94	
45	4.85		4.53	4.73	
46	4.71		4.67	4.93	
47	4.34	4.06	4.12	4.40	
48	4.05	4.67	4.77	4.83	
49	3.67	4.12	4.18	4.36	
50	3.78	4.58	4.62	4.82	
51	4.19	4.27	4.57	4.53	
52	3.83	4.68	4.78	4.97	
53	3.86	3.97	4.07	4.21	
54	4.59	4.59	4.61	4.83	
55	4.02	4.26	4.36	4.40	
56	4.36	4.62	4.76	4.94	
57	4.20	4.18	4.28	4.48	
58	4.09	4.52	4.56	4.74	
59	4.09		4.28	4.46	
60	4.12		4.49	4.81	
61	4.20		4.33	4.41	
62	4.45		4.25	4.77	
63	4.33		4.09	4.37	
64	4.62		4.33	4.99	
Mean,	4.33	4.34	4.38	4.58	4.63

In the following table will be found the percentage of milk sugar obtained by using varying quantities of the mercuric iodide reagent,

and a comparison of the results obtained with those given by the use of acid mercuric nitrate and basic plumbic acetate.

TABLE III.

Reagents Employed.

No.	PbA	MR	Mercuric Iodide.			
			20 cc.	26 cc.	30 cc.	35 cc.
			Percentage of Milk Sugar.			
1	4.28	4.48	4.56	4.56		
2	4.46	4.57	4.62	4.66		
3	4.37	4.65	4.63	4.63	4.60	4.65
4	4.37	4.53	4.60	4.53	4.63	4.60
5	4.38	4.63	4.50	4.53	4.53	4.59
6	4.33	4.67	4.43	4.53	4.60	4.66
7	4.30	4.67	4.67	4.67	4.59	4.57
8	4.33	4.59	4.50	4.53	4.50	4.59
9	4.27	4.60	4.63	4.60	4.66	4.66
	4.34	4.60	4.57	4.58	4.61	4.62

Albumen remaining in Filtrate from Lead Acetate and Mercuric Iodide Solutions.

From the fact that the polariscopic readings show that solutions of milk prepared with lead acetate have a lower rotating power than those prepared with mercury salts, it is to be inferred that the lead reagent either leaves certain soluble and transparent kinds of albumen in solution, or else dissolves a portion of those which are at first precipitated. To test the accuracy of this supposition a few analyses were made to determine the amount of albumen left in the filtrate from the lead and mercury reagents. At the same time different quantities of the mercuric iodide solution were used, in order to determine the amount which would give the best results. For 60 cc. milk the quantity of mercuric iodide to be used should be 25 to 30 cc.

In the following table will be found the percentages of albumen in the whey after precipitating with the reagents noted and filtering. Ten cc. of the filtrate were evaporated to dryness in a thin glass dish, and the dried residue (with the glass) burned with soda lime. The calculated nitrogen was then multiplied by 6.25 and the product taken as the percentage of albumen.

TABLE IV.

Per cent. albumen in filtrate from PbA.	Per cent. albumen in filtrate from HgI ₂ . 15 cc.	Per cent. albumen in filtrate from HgI ₂ . 20 cc.	Per cent. albumen in filtrate from HgI ₂ . 25 cc.	Per cent. albumen in filtrate from HgI ₂ . 30 cc.	Per cent. albumen in filtrate from HgI ₂ . 35 cc.
.0865	.195	.0865	.0865	.0562	.0865
.113	.0674	.0865	.0865	.0865	.0865
.113	.0674	.0562	.113	.0562	.0312
.0865	.0674	.0562	.113	.0562	.0562
.113	.0674	.0562	.0312	.0865	.0562
.113	.009	.0865	.03	.0862	.1412
.113		.1412	.113	.1412	.0562
.195		.1412	.113	.1412	.1412
.113		.1412	.0865	.1412	.0865
.1412		.113	.1412	.113	.0865
.113		.1362	.009		
		.125			
		.009			
		.009			
Mean, .1182	.0789	.0888	.0839	.0964	.0828

In table No. V will be found percentages of albumen remaining in filtrate from lead acetate precipitation of forty-two samples taken from those represented in table No. II. From these two tables it is at once seen that the quantity of laevo-rotatory matter remaining in milk after treatment with basic lead acetate is much greater than in those samples treated with the two mercuric salts. This explains at once the higher per cent. of milk sugar obtained by using the last-named reagents, and shows that the use of lead acetate as a clarifying agent must be abandoned.

Per cent. Albumen after Precipitation by Lead Acetate.

No.	Pr ct.	No.	Pr ct.	No.	Pr ct.
1	.250	15	.271	29	.350
2	.306	16	.237	30	.374
3	.135	17	.237	31	.329
4	.272	18	.169	32	.305
5	.134	19	.103	33	.305
6	.239	20	.271	34	.237
7	.301	21	.237	35	.305
8	.305	22	.271	36	.339
9	.237	23	.235	37	.237
10	.339	24	.271	38	.374
11	.271	25	.237	39	.203
12	.305	26	.237	40	.373
13	.267	27	.271	41	.305
14	.237	28	.339	42	.339

Mean, .278

Comparison of Results obtained by Extraction with Alcohol and Polarisation.

By consulting table II it will be seen that the percentage of sugar obtained by extraction with alcohol is practically the same as that got by polarisation of the lead acetate filtrate.

Thus, the mean percentage of sugar by alcohol (65 analyses) is 4.32; by lead acetate, cold (53 analyses) is 4.34; by lead acetate, hot (64 analyses) is 4.38; by mercuric nitrate, cold (61 analyses) is 4.58; by mercuric nitrate, hot (24 analyses) is 4.63.

If now the milk sugar, as has already been intimated, exists in an anhydrous state after extraction with alcohol, the percentage of it after the addition of the molecule of water would be increased. Thus molecular weight of anhydrous milk sugar 342 : mol. wt. of the hydrous from $360 = 4.38 : x$, whence the value of $x = 4.61$. This agrees very nearly with the number obtained by acid mercuric nitrate.

By a study of table V it is found that mercuric iodide gives nearly the same rotatory power as mercuric nitrate, and also that by combustion the filtrates from the milks clarified by lead acetate contain more albumen than those prepared with mercuric iodide. There is, therefore, every reason for believing that the numbers given by the mercury salts are nearer the truth than those from the lead.

It may be urged that the increased rotatory power observed by the mercury salts is due to the conversion, by the dilute acids, of a part of the lactose into galactose, which has a rotatory power greater than that of milk sugar. But when it is remembered that the quantity of acid introduced is extremely minute, that the samples need not be warmed, that they can be filtered and polarised within a few minutes of the time of the introduction of the reagents, the suggestion is seen to be of no force.

For example, in the acid mercuric nitrate it was found that the percentage of sugar was the same whether one, five or ten cc. of the reagent were employed, and whether it was polarised immediately or after heating and cooling. It is evident that one cc. of the reagent, containing less than a half cc. of nitric acid and diluted in 100 cc. of liquid, could not exert any notable effect on the rotatory power of the solution.

In the mercuric iodide solution 20 cc. of acetic acid are used for every 660 cc. of the reagent.

Thirty cc. of this reagent contain, therefore, about one cc. of acid. This in 100 cc. of liquid, immediately filtered and polarised, could not affect in any marked degree the rotatory power.

Since combustion with soda lime shows that the filtrate from the mercuric iodide sample is practically free from albumen, it is evident that the numbers obtained in this way must be a near approximation to the truth.

The Process of Analysis.

The reagents, apparatus and manipulation necessary to give the most reliable results in milk sugar estimation are as follows :

Reagents.—1. *Basic plumbic acetate*, sp. gr. 1.97. Boil a saturated solution of sugar of lead with an excess of litharge, and make it of the strength indicated above. One cubic centimetre of this will precipitate the albumens in 50 to 60 cc. of milk.

2. *Acid mercuric nitrate* dissolves mercury in double its weight of nitric acid, sp. gr. 1.42. Add to the solution an equal volume of water. One cubic centimetre of this reagent is sufficient for the quantity of milk mentioned above. Larger quantities can be used without affecting the results of polarisation.

3. *Mercuric iodide with acetic acid* (composition already given).

Apparatus.

1. Pipettes marked at 59.5, 60 and 60.5 cc.
2. Sugar flasks marked at 102.4 cc.
3. Filters, observation tubes and polariscope.
4. Sp. gr. spindle and cylinder.
5. Thermometers.

Manipulation.

1. The room and milk should be kept at a constant temperature. It is not important that the temperature should be any given degree. The work can be carried on equally well at 15°, 20° or 25°. The slight variations in rotatory power within the above limits will not affect the result for analytical purposes. The temperature selected should be the one which is most easily kept constant.

2. The sp. gr. of milk is determined. For general work this is done by a delicate sp. gr. spindle. Where greater accuracy is required use sp. gr. flask.

3. If the sp. gr. be 1.026 or nearly so, measure out 60.5 cc. into the sugar flask. Add 1 cc. of mercuric nitrate solution or 30 cc. mercuric iodide solution and fill to 102.4 cc. mark. The precipitated albumen occupies a volume of about 2.4 cc. Hence the solution is really 100 cc. If the sp. gr. is 1.030 use 60 cc. of milk. If sp. gr. is 1.034 use 59.5 cc. milk.

4. Fill up to mark in 102.4 cc. flask, shake well, filter and polarise.

Notes.

In the above method of analysis the specific rotatory power of milk sugar is taken at 52.5, and the weight of it in 100 cc. solution to read 100 degrees in the cane sugar scale at 20.56 grams. This is for instruments requiring 16.19 grams sucrose to produce a rotation of 100 sugar degrees. It will be easy to calculate the number for milk sugar whatever instrument is employed.

Since the quantity of milk taken is three times 20.56 grams, the polariscopic readings divided by 3 give at once the percentage of milk sugar when a 200 mm. tube is used.

If a 400 mm. tube is employed, divide reading by 6; if a 500 mm. tube is used, divide by 7.5.

Since it requires but little more time, it is advisable to make the analysis in duplicate, and take four readings for each tube. By following this method gross errors of observation are detected and avoided.

By using a flask graduated at 102.4 for 60 cc. no correction for volume of precipitated caseine need be made. In no case is it necessary to heat the sample before polarising.

